UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

October 29, 2008

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 72977-3 Axen (R) 30;
DP Barcode: 356116

From: Tajah L. Blackburn, Ph.D., Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Michele Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510P)

To: Marshall Swindell PM 33/ Martha Terry
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Pure Bioscience
1725 Gillespie Way
El Cajon, CA 92020

Formulations from Label

Active Ingredient(s)	% by wt.
Silver[1]	0.003%
Citric Acid	4.846%
Other Ingredients	95.151%
Total	100.000%

I BACKGROUND

The product, Axen 30 (EPA Reg. No. 72977-3), is a registered, ready-to-use disinfectant (bactericide, fungicide, virucide) and deodorizer for use on hard, non-porous surfaces in household, commercial, institutional, industrial, food preparation, animal care, and hospital or medical environments. In response to questions raised under the Agency's Antimicrobial Testing Program (ATP), the applicant conducted additional testing of its product. This testing was for all microorganisms identified on the product label with a 30-second exposure time, specifically *Pseudomonas aeruginosa*, *Salmonella enterica*, *Staphylococcus aureus*, *Listeria monocytogenes*, and Human immunodeficiency virus type 1. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121; and MicroBioTest, Inc., located at 105 Carpenter Drive in Sterling, VA 20164.

This data package contained a letter from the applicant's representative to EPA (dated August 25, 2008), EPA Form 8570-1 (Application for Pesticide), three studies (MRID 475200-01 through 475200-03), Statements of No Data Confidentiality Claims for all three studies, and the proposed label.

II USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces, including: activity centers, appliances, bed frames, cabinets, chairs, child car seats, computer keyboards, counters, cribs, desks, diaper changing tables, diaper pails, doorknobs, examination tables, exterior toilet and urinal surfaces, faucet handles, floors, grocery carts, hand rails, jungle gyms, lab benches, laundry hampers, light switch covers, patio furniture, play houses, playpens, sinks, strollers, showers, tables, tanning beds, telephones, toy boxes, toys, tubs, walls, waste containers, and wheelchairs. The label indicates that the product may be used on hard, non-porous surfaces including: glass, glazed porcelain, glazed tiles, metal, painted surfaces, and plastic. Directions on the proposed label provided the following information regarding use of the product:

> As a disinfectant against bacteria: Pre-clean surfaces. Wet surfaces completely with the product. Surfaces must remain wet for 2 minutes. Wipe surfaces dry with a clean towel if desired.
>
> As a disinfectant against Human immunodeficiency virus type 1: Pre-clean surfaces. Wet surfaces completely with the product. Surfaces must remain wet for 30 seconds. Wipe surfaces dry with a clean towel if desired.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

<u>Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments</u>

The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested

with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old, against *Salmonella enterica* (ATCC 10708; formerly *Salmonella choleraesuis*), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). To support products labeled as "disinfectants," killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria)

Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Products Controlling Microorganisms of Economic or Aesthetic Significance

Algaecides, slimicides, preservatives, deodorizers, and other products expressly claiming control of microorganisms of economic or aesthetic significance not directly related to human health do not require efficacy data. However, adequate dosage recommendations and complete directions for use must be provided in labeling.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 475200-01 "AOAC Germicidal Spray Method," for Axen 30, Test Organisms: *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 6538), and *Salmonella enterica* (ATCC 10708), by Becky Lien. Study conducted at ATS Labs. Study completion date – August 21, 2008. Project Number A06557.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 6538), and *Salmonella enterica* (ATCC 10708). Three lots (Lot Nos. P08142002, P08189001, and P08191001) of the product, Axen 30, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. At least one of the product lots tested (i.e., Lot No. P08142002) was at least 60 days old at the time of testing. The product was received ready-to-use, as a trigger spray. Testing was conducted on July 27, 2008, July 28, 2008, July 31, 2008, and August 11, 2008. Cultures of the challenge microorganisms were prepared in accordance with the published AOAC methods, with the following exceptions: (1) the *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Staphylococcus aureus* cultures were incubated for 48-54 hours (which is a greater amount of time than the 18-24 hours specified in the AOAC methods), and (2) the pellicle was aspirated from the *Pseudomonas aeruginosa* culture on the day of use (which deviates from the AOAC method instruction not to disturb the pellicle). The product was not tested in the presence of a 5% organic soil load. Sixty (60) glass slide carriers were inoculated with 0.01 mL of a 48-54 hour old suspension of the test organism. Inoculum was uniformly spread over the entire surface of the glass slide carriers (18 mm x 36 mm), an area comparable to the AOAC method specified 1 inch x 1 inch area. The carriers were dried for 30 minutes at 35-37°C at 39-44% relative humidity (which is a slightly cooler temperature than the 37°C specified in the AOAC method). For each lot of product, separate carriers were sprayed (2 pumps) with the product at a distance of 6-8 inches from the carrier surface. Different carriers were exposed to the product for 30 seconds and 120 seconds at 22-23°C at 44-53% relative humidity. Following the exposure period, the remaining liquid was drained from the carrier. Individual carriers were transferred to 20 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. All subcultures were incubated for 44-47 hours at 35-37°C (which is a shorter time than the 48 hours specified in the AOAC method and a slightly cooler temperature than the 37°C specified in the AOAC method). For testing conducted on July 31, 2008, the subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation or incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: Testing conducted on July 31, 2008 against *Staphylococcus aureus* at a 30-second exposure time showed growth in subcultures of 2 of the 60 carriers for Lot No. P08191001. Testing conducted on July 31, 2008 against *Salmonella enterica* at a 30-second exposure time showed growth in subcultures of 2 of the 60 carriers for Lot No. P08189001. Testing was repeated to test for false positive.

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2. MRID 475200-02 "AOAC Germicidal Spray Method," for Axen 30, Test Organism: *Listeria monocytogenes* (ATCC 19111), by Becky Lien. Study conducted at ATS Labs. Study completion date – August 5, 2008. Project Number A06558.

This study was conducted against *Listeria monocytogenes* (ATCC 19111). Two lots (Lot Nos. P08189001 and P08191001) of the product, Axen 30, were tested using the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. The product was received ready-to-use, as a trigger spray. A culture of the challenge microorganism was prepared in accordance with the published AOAC method, with the following exception: the culture was incubated for 48-54 hours (which is a greater amount of time than the 18-24 hours specified in the AOAC method). The product was not tested in the presence of a 5% organic soil load. Ten (10) glass slide carriers were inoculated with 0.01 mL of a 48-54 hour old suspension of the test organism. Inoculum was uniformly spread over the entire surface of the glass slide carriers (18 mm x 36 mm), an area comparable to the AOAC method specified 1 inch x 1 inch area. The carriers were dried for 30 minutes at 36.0°C at 40% relative humidity (which is a slightly cooler temperature than the 37°C specified in the AOAC method). For each lot of product, separate carriers were sprayed (2 pumps) with the product at a distance of 6-8 inches from the carrier surface. Different carriers were exposed to the product for 30 seconds and 120 seconds at 23°C at 54% relative humidity. Following the exposure period, the remaining liquid was drained from the carrier. Individual carriers were transferred to 20 mL of Brain Heart Infusion Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. Carriers were transferred from the primary subcultures into individual secondary subcultures containing 20 mL of Brain Heart Infusion Broth at least 30 minutes following the first transfer. All subcultures were incubated for 44 hours at 35-37°C (which is a shorter time than the 48 hours specified in the AOAC method and a slightly cooler temperature than the 37°C specified in the AOAC method). Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

3. MRID 475200-03 "Virucidal Efficacy Test Using Human Immunodeficiency Virus Type 1" for Axen 30, by Lauren A. Blaszak. Study conducted at MicroBioTest, Inc. Study completion date – April 30, 2008. Project Identification Number 616-103.

This study was conducted against Human immunodeficiency virus type 1 (HIV-1; obtained from ZeptoMetrix Corporation), using C8166 cells (obtained from the University of Pennsylvania) as the host system. Two lots (Lot Nos. P08077001 and P08077002) of the product, Axen 30, were tested according to MicroBioTest Protocol "Virucidal Efficacy Test Using Human Immunodeficiency Virus Type 1," dated March 14, 2008 (copy provided). The product was received ready-to-use. The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were allowed to dry at ambient temperature. For each lot of product, separate dried virus films were sprayed with the product at a distance of 4 inches from the carrier surface until thoroughly wet. Each carrier remained exposed to the product for 30 seconds at 25-26°C. Following exposure, the plates were neutralized with 2.0 mL of newborn calf serum with 50% HEPES. The plates were scraped with a cell scraper to

re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephacryl columns, and diluted serially in RPMI 1640 with 10% fetal bovine serum. C8166 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 9-12 days at 36±2°C in 5±1% CO_2. The plates were re-fed as necessary. Following incubation, the cultures were examined microscopically for the presence of infectious virus. Controls included those for cell viability, column titer count, plate recovery count, cytotoxicity, and neutralization effectiveness/viral interference. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

V RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Population (CFU/ Carrier)
		Lot No. P08142002	Lot No. P08189001	Lot No. P08191001	
30-Second Exposure Time					
475200-01	*Pseudomonas aeruginosa* Test Date: 7/27/2008	0/60	0/60	0/60	3.9×10^6
475200-01	*Staphylococcus aureus* Test Date: 7/31/2008 Test Date: 8/11/2008	1/60 ---	0/60 ---	2/60 5/60	1.87×10^6 1.64×10^6
475200-01	*Salmonella enterica* Test Date: 7/31/2008 Test Date: 8/11/2008	1/60 ---	2/60 1/60	0/60 ---	1.22×10^5 9.8×10^4
475200-02	*Listeria monocytogenes*	---	1°=3/10 2°=3/10	1°=2/10 2°=2/10	2.05×10^6
120-Second Exposure Time					
475200-01	*Pseudomonas aeruginosa* Test Date: 7/27/2008	0/60	0/60	0/60	3.9×10^6
475200-01	*Staphylococcus aureus* Test Date: 7/31/2008	0/60	1/60	1/60	1.87×10^6
475200-01	*Salmonella enterica* Test Date: 7/28/2008	0/60	0/60	0/60	1.68×10^5
475200-02	*Listeria monocytogenes*	---	1°=0/10 2°=0/10	1°=0/10 2°=0/10	2.05×10^6

MRID	Organism	Results	Plate

Number			Lot No. P08077001	Lot No. P08077002	Recovery Control
475200-03	Human immunodeficiency virus type 1	10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{6.18}$ $TCID_{50}$/mL
		$TCID_{50}$/ mL	$\leq 10^{2.83}$	$\leq 10^{2.83}$	

VI CONCLUSIONS

1. The submitted efficacy data (MRID 475200-01) support the use of the product, Axen 30, as a disinfectant with bactericidal activity against *Pseudomonas aeruginosa* and *Salmonella enterica* on pre-cleaned, hard, non-porous surfaces for a 30-second contact time. Killing was observed in the subcultures of at least 59 of the 60 carriers tested against the required number of product lots. [Note that repeat testing was conducted on one product lot against *Salmonella enterica* to evaluate for false positives.] At least one of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

2. The submitted efficacy data (MRID 475200-01 and 475200-02) support the use of the product, Axen 30, as a disinfectant with bactericidal activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella enterica*, and *Listeria monocytogenes* on pre-cleaned, hard, non-porous surfaces for a 120-second contact time. Acceptable killing was observed in the required number of subcultures tested against the required number of product lots. In testing against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella enterica*, at least one of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

3. The submitted efficacy data (MRID 475200-01 and 475200-02) do not support the use of the product, Axen 30, as a disinfectant with bactericidal activity against *Staphylococcus aureus* and *Listeria monocytogenes* on pre-cleaned, hard, non-porous surfaces for a 30-second contact time. Acceptable killing was not observed in the required number of subcultures against the required number of product lots.

4. The submitted efficacy data (MRID 475200-03) support the use of the product, Axen 30, as a disinfectant with virucidal activity against Human immunodeficiency virus type 1 on hard, non-porous surfaces in the presence of at least a 5% organic soil load for a 30-second contact time. A recoverable virus titer of at least 10^4 was achieved. Complete inactivation (no growth) was indicated in all dilutions tested.

VII RECOMMENDATIONS

1. The proposed label claims are **unacceptable** regarding the use of the Axen 30, as a disinfectant effective disinfectant against *Pseudomonas aeruginosa*, *Salmonella*

enterica, and *Staphylococcus aureus* on pre-cleaned, hard, non-porous surfaces for a 30 second contact time. Efficacy was not demonstrated at the 30-second contact time for *Staphylococcus aureus*, therefore the claim is not acceptable for *Salmonella enterica* and *Pseudomonas aeruginosa*, as these are the disinfectant qualifying bacteria. **The acceptable claim for *Staphylococcus aureus*, *Salmonella enterica*, and *Pseudomonas aeruginosa* is the 120-second contact time (2 minutes) on pre-cleaned, hard, non-porous surfaces.**

2. The proposed label claim is acceptable regarding the use of the product, Axen 30, as a disinfectant effective virucide against Human immunodeficiency virus type 1 on pre-cleaned, hard, non-porous surfaces for a 30-second contact time. These claims are supported by the submitted data.

3. The proposed label claim is acceptable regarding the use of the product, Axen 30 as an effective disinfectant against *Listeria monocytogenes* on pre-cleaned, hard, non-porous surfaces for a 2-minute contact time. This claim is supported by the submitted data.

4. The proposed label claims are acceptable regarding use of the product, Axen 30, as a disinfectant for use on hard, non-porous surfaces against the following microorganisms for the listed contact times:

Acinetobacter baumannii	2 minutes
Campylobacter jejuni	2 minutes
Staphylococcus aureus – CA-MRSA	2 minutes
Staphylococcus aureus – CA-MRSA, PVL Positive	2 minutes
Avian Influenza A	10 minutes
Human Coronavirus	3 minutes
Norovirus (as Feline calicivirus)	10 minutes
Rotavirus	3 minutes

Data provided in September 2007 to support these claims.

5. The proposed label identifies a new active concentration of citric acid (i.e., 4.846%). The data package did not include information regarding this change. The active concentration of citric acid on the last accepted label (dated October 5, 2006) was "4.840%."

6. The proposed label states that the product can be used as a deodorizer. The label must be revised to provide adequate dosage recommendations and complete directions for use of the product as a deodorizer.

7. The medical device use claim on the proposed label differs slightly from the statement set forth in PR Notice 94-4. The medical device use claim on the proposed label must be revised to read: "This product is not to be used as a terminal sterilant/high-level disinfectant on any surface or instrument that (1) is introduced directly into the human body, either into or in contact with the bloodstream or normally sterile areas of the body, or (2) contacts intact mucous membranes but which does not ordinarily penetrate the blood barrier or otherwise enter normally sterile areas of the

body. This product may be used to pre-clean or decontaminate critical or semi-critical medical devices prior to sterilization or high-level disinfection."

8. Under the "Storage" section of the proposed label, provide instructions for storing the product. Guidance on developing appropriate pesticide storage instructions can be found at http://www.epa.gov/oppfead1/labeling/lrm/, Chapter 13 (Storage and Disposal).

9. Numerous requests (dating back to 2004, and 2006) have been made for the removal of the residual activity for up to 24 hours after initial application claim. Provide the Agency with resolution document for retention of label claim.